S93	1	S90 and tetO	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/16 17:31
S94	6377	(tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S95	144476	promoter or terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S96	3742	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S97	42838	(promoter or terminator) SAME (tissue specific)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S98	2309	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and ((promoter or terminator) SAME (tissue specific))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S99	0	adpgk WITH tet	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S100	102	"protein IX" SAME adenoviral	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S101	63	("protein IX" SAME adenoviral) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S102	2530	Tn10 or "tetracycline operon"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S103	7	adenovrial and ("gene regulation" or "gene activity" or "gene expression")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S104	33170	adenovirus	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S105	27637	gene WITH (express? or regulat? or activ?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S106	302	(Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S107	301	((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S108	147	(((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S109	0	tetracyline WITH "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S110	0	tetracyline SAME "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S111	601	tet-off or "tet off"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S112	2	((((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)) and (tet-off or "tet off")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S113	9	reeves.in. and "retroviral"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S114	4035	tyrosine and hydroxylase	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S115	19044	cmv and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S116	47922	tet or tetracycline or "tet operon" or operon	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S117	1011	(tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S118	1	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and adenovir?	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S119	145	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and pgk	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S120	3	"upstream mouse sequence"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S121	8	"6552003".pn. or "6432701".pn. or "6632427".pn. or "6756523".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S122	18262	PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S123	1333	(PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S124	764	((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S125	602	(((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S126	168	((((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)) and (terminator or silenc?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S127	6377	(tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S128	144476	promoter or terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S129	3742	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S130	42838	(promoter or terminator) SAME (tissue specific)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S131	2309	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and ((promoter or terminator) SAME (tissue specific))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S132	0	adpgk WITH tet	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S133	102	"protein IX" SAME adenoviral	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S134	2530	Tn10 or "tetracycline operon"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S135	33170	adenovirus	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S136	27637	gene WITH (express? or regulat? or activ?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S137	302	(Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S138	301	((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S139	0	tetracyline WITH "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S140	0	tetracyline SAME "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S141	601	tet-off or "tet off"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S142	4035	tyrosine and hydroxylase	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S143	19044	cmv and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S144	47922	tet or tetracycline or "tet operon" or operon	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S145	1011	(tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S146	1	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and adenovir?	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S147	18262	PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S148	1333	(PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S149	764	((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S150	602	(((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S151	63	("protein IX" SAME adenoviral) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S152	7	adenovrial and ("gene regulation" or "gene activity" or "gene expression")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S153	2	((((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)) and (tet-off or "tet off")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S154	9	reeves.in. and "retroviral"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S155	3	"upstream mouse sequence"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S156	8	"6552003".pn. or "6432701".pn. or "6632427".pn. or "6756523".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S157	147	(((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S158	145	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and pgk	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S159	168	((((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)) and (terminator or silenc?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S160	8632054	"WO" (s) "20463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S161	3	"WO 97/20463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32

S162	1	"WO 98/37185"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S163	0	"WO98/37185"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S164	0	"PCT/US98/03092"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S165	0	"US98/03092"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S166	19752	xu.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S167	3	S166 and "controlled gene expression"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S168	2	"9720463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S169	0	S167 and (nonviral or non-viral)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:32
S170	7520326	(cell-specific or tissue-specific) (s) promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S171	2	S167 and S170	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S172	35964	"cell specific" or "tissue specific"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S173	0	S172 and S167	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33

S174	2	"5464758".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S175	63	kaleko.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S176	4	S175 and "fiber shaft"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S177	2	"5650298".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S178	0	S177 and ("protein of interest" or "neurotransmitter" or "trophic factor")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S179	0	S177 and "target protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S180	0	S177 and "gene of interest"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S181	1	S177 and "interest"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S182	188	"l1" and "nerve cell"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S183	2	"5650298".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S184	0	S183 and "nerve cell"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S185	0	S183 and "nerve"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33

S186	1	S183 and tetO	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:33
S187	487	mallet.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 13:37
S188	10	S187 and tetracycline	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 15:33
S189	2531	tetracycline SAME doxycycline	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:12
S190	432	S189 and CMv	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:12
S191	0	S190 and terminatory	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:12
S192	130	S190 and terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:12
S193	2347	"tyrosine hydroxylase"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:13
S194	38	S192 and S193	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:59
S195	76	ttA and tetop	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:13
S196	3	S194 and S195	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:13
S197	46	unidirectional WITH transcription	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:19

S198	0	S197 and S189	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:19
S199	149	"SAME orientation" with transcription	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:20
S200	0	S199 and S189	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:20
S201	0	S194 and CMNath & Associates	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:59
S202	38	S194 and CMv	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 16:59
S203	2531	tetracycline SAME doxycycline	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S204	432	S203 and CMv	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S205	130	S204 and terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S206	2347	"tyrosine hydroxylase"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S207	38	S205 and S206	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S208	38	S207 and CMv	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50
S209	38	S208 and vector	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/06/01 17:50

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 FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 14:36:34 ON 09 JUN 2005
L1         12887 S MALLET?/AU OR CORTI?/AU
L2           350 S MCGEADY?/AU
L3           633 S PGK (P) PROMOTER
L4             1 S TETRACYLINE (P) OPERATOR
L5           242 S UMS OR "UPSTREAM MOUSE SEQUENCE"
L6             3 S "PHTS3MS"
L7           189 S "TTA" AND "TET"
L8           119 S "TETRACYCLINE REGULATED SYSTEM"
L9          3850 S CMV (P) PROMOTER
L10          443 S BUJARD?/AU
L11         1189 S TET (P) (OPERON OR PROMOTER OR "ON SYSTEM" OR ACTIVATOR)
L12       251226 S HIS
L13            0 S L1 AND L3
L14            0 S L1 AND L5
L15        13680 S L1 OR L2 OR L10
L16           37 S L15 AND L11
L17           21 S L16 NOT PY>=2002
L18           11 DUP REM L17 (10 DUPLICATES REMOVED)
L19       503974 S FIRST AND SECOND
L20         5841 S L19 (P) PROMOTER
L21            0 S L20 AND L8
L22            8 S L15 AND L20
L23            4 DUP REM L22 (4 DUPLICATES REMOVED)
L24            0 S L5 AND L8
L25            0 S L7 AND L5
L26           63 S TETOP OR (TET (2W) OPERON)
L27            0 S L26 AND L9
L28            0 S L26 AND L19
L29            0 S L26 AND L3
L30            0 S L26 AND UM
L31            0 S L26 AND L1
L32            0 S L26 AND CMV
L33            0 S UTR (P) NONNATIVE
L34            0 S UTR AND NONNATIVE
L35            0 S UTR AND NON-NATIVE
L36        24262 S DHFR OR PGK OR EF1A OR BGLOBIN OR MHCA
L37           11 S L36 AND L15
L38            5 DUP REM L37 (6 DUPLICATES REMOVED)
L39          240 S TTA (S) PROMOTER
L40           78 S L39 NOT PY>=1999
L41           28 DUP REM L40 (50 DUPLICATES REMOVED)
L42       505575 S EXPRESSION (S) REGULAT?
L43           21 S L41 AND L42
L44            0 S "SECOND PROMOTER" AND L43
L45            0 S "ADDITIONAL PROMOTER" AND L43
L46            0 S "DUAL PROMOTER" AND L39
L47          182 S "DUAL PROMOTER"
L48            5 S L43 AND TET
L49            5 DUP REM L48 (0 DUPLICATES REMOVED)

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L49 ANSWER 1 OF 5 MEDLINE on STN
ACCESSION NUMBER: 97440934 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9296393
TITLE: Tetracycline **regulated expression** of vimentin in fibroblasts derived from vimentin null mice.
AUTHOR: Holwell T A; Schweitzer S C; Evans R M
CORPORATE SOURCE: Department of Pathology, University of Colorado Health Sciences Center, Denver 80262, USA.
CONTRACT NUMBER: HL51850 (NHLBI)
SOURCE: Journal of cell science, (1997 Aug) 110 (Pt 16) 1947-56.
Journal code: 0052457. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971024
Last Updated on STN: 19971024
Entered Medline: 19971016

AB Fibroblast cell lines were derived from vim-/- mice that express a mouse vimentin transgene in a tetracycline regulatable manner. Vimentin null mouse primary embryo fibroblasts were transformed with SV-40 early genes and vim- cell lines were isolated. A vim- cell line was then serially transfected with an **expression** plasmid encoding the tetracycline **regulatable** transactivator (**tTA**) and a mouse vimentin cDNA **expression** plasmid under the **regulation** of Escherichia coli **tet** operator and minimal CMV **promoter** sequences. Two stable cell lines were obtained that contained little or no vimentin in the presence of low concentrations of tetracycline but rapidly expressed abundant vimentin filaments after removal of tetracycline. The vimentin content of one cell line was similar to that of control vim+/+ fibroblasts. The level of transgene **expression** could be **regulated** by the concentration of tetracycline in a dose dependent fashion. Induction of vimentin expression in these cells did not observably affect cell growth, the distribution of microfilaments or microtubules, or the shape of the nucleus. Enucleation studies indicated that while disassembly of microfilaments significantly increased the sensitivity of the cells to enucleation, the presence or absence of vimentin had no detectable effect on the degree of enucleation with increasing sedimentation force. Monolayer wounding experiments demonstrated that vimentin expression did not alter the mobility of polarized cells at the edge of the wound. Experiments to more directly test the effect of vimentin expression on the capacity of these fibroblasts to survive mechanical trauma indicated that vimentin expression had no obvious effect on the survival of suspension cells subjected to nitrogen cavitation or the fraction of cells that survived the mechanical scraping of monolayer culture. These studies indicate that vimentin expression in a single population of cells does not have an obvious effect on cytoplasmic organization and provides a useful system to study the effects of IFs on the capacity of individual cells to resist mechanical injury.

L49 ANSWER 2 OF 5 MEDLINE on STN
ACCESSION NUMBER: 97168989 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9016796
TITLE: Adenovirus-mediated inducible gene expression through tetracycline-controllable transactivator with nuclear localization signal.
AUTHOR: Yoshida Y; Hamada H
CORPORATE SOURCE: Department of Molecular Biotherapy Research, Cancer Chemotherapy Center, Cancer Institute, Toshima-ku, Tokyo, Japan.
SOURCE: Biochemical and biophysical research communications, (1997 Jan 13) 230 (2) 426-30.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 19970313
Entered Medline: 19970305

AB Tetracycline-controllable expression vectors are widely used for inducible expression in mammalian cells. The limitation of this system is the difficulty in expressing high levels of the chimeric transactivator tTA. In this study, we demonstrate a utility of recombinant adenoviruses for the tetracycline-controllable expression system. Unexpectedly, the original **tTA** transactivator did not show sufficient **regulation** of the reporter gene **expression** driven by the tetracycline-responsive **promoter** (**Tet**). By adding the nuclear localization signal on the tTA transactivator (NtTA), we achieved tight **regulation** and high-level induction of the reporter gene **expression**. The NtTA driven by various promoters demonstrated strict tetracycline controllability at 1 microg/ml of tetracycline and above. The methodology for adenovirus-mediated inducible gene expression has wide applicability. Controllable expression of cytotoxic viral proteins will be applicable for antiviral vaccine productions and pseudotype viral vector generations.

L49 ANSWER 3 OF 5 MEDLINE on STN
ACCESSION NUMBER: 97345779 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9202271
TITLE: **Regulated expression** of the diphtheria toxin A gene in human glioma cells using prokaryotic transcriptional control elements.
AUTHOR: Paulus W; Baur I; Oberer D M; Breakefield X O; Reeves S A
CORPORATE SOURCE: Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, USA.
CONTRACT NUMBER: NS24279 (NINDS)
SOURCE: Journal of neurosurgery, (1997 Jul) 87 (1) 89-95.
Journal code: 0253357. ISSN: 0022-3085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970722

AB Because accurate **regulation** of toxin gene **expression** is critical for safe and effective gene therapy applications, the authors have examined the **regulation** of diphtheria toxin A (DTA) fragment **expression** in human glioma cell lines using two transcriptional control systems derived from Escherichia coli: the tetracycline (**Tet**) system and the lactose (Lac) system. The **Tet** system includes a tetracycline-controlled transactivator (**tTA**), a **tTA**-responsive minimum human cytomegalovirus (hCMV) **promoter** controlling the expression of the DTA gene, and tetracycline as an allosteric inhibitor. The Lac system includes the lac repressor (lacR), a lacR-**regulated** Rous sarcoma virus-long terminal repeat (RSV-LTR) promoter controlling the **expression** of the DTA gene, and isopropyl-thio-beta-D-galactoside (IPTG) as an allosteric inducer. Expression plasmids encoding either tTA or lacR were transfected into U-87MG and U-343MG glioma cells along with the responsive DTA plasmid. Cell killing was monitored by the ability of the toxin to abolish protein synthesis and was quantitated using a luciferase reporter gene. In the **Tet** system, tumor cell killing could be regulated by tetracycline up to 120-fold. In contrast, only a twofold IPTG-dependent **regulation** was obtained using the Lac system because of an incomplete repression of DTA **expression** in the uninduced state. Replacement of the RSV-LTR promoter with the heavy metal-inducible mouse metallothionein-1 promoter in the lacR-responsive unit, as well as the generation of a clonal glioma cell line expressing lacR, did not significantly enhance regulation of DTA in the Lac system. In conclusion, this study demonstrates that the **Tet** system is of potential use in gene therapy applications in which **regulated**

expression of a therapeutic gene is an important issue.

L49 ANSWER 4 OF 5 MEDLINE on STN
ACCESSION NUMBER: 96269409 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8682308
TITLE: Controlled gene **expression** in mammalian cells via a **regulatory** cascade involving the tetracycline transactivator and lac repressor.
AUTHOR: Aubrecht J; Manivasakam P; Schiestl R H
CORPORATE SOURCE: Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA 02115, USA.
SOURCE: Gene, (1996 Jun 26) 172 (2) 227-31.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960828
Last Updated on STN: 19960828
Entered Medline: 19960822

AB Regulatory cascades or regulons control pathways at multiple points or multiple genes by one initial signal. In this paper, we describe the construction of an artificial regulatory cascade in CHO cells, which responded to various concentrations of tetracycline (Tc) and/or IPTG. The system consists of the constitutively produced transactivator (**TTA**) of the Tc operon (**tet**), which induced the expression of a lacI gene controlled by **tet** operator (tetO) and upstream CMV **promoter** (p*CMV) sequences. LacI repressed the activity of a cat gene by binding to lacO sites in its upstream RSV promoter (pRSV) region. However, this repression could be alleviated by exposure to Tc or IPTG, which inhibited the binding activities of TTA and LacI, respectively. Hence, treatment with either Tc or IPTG led to a tenfold increase in CAT activity. After the withdrawal of the inducer, cat expression reverted to basal levels. Regulation by Tc showed a phenotypic lag, and full induction was reached after 192 h, whereas IPTG addition led to full induction within 24 h. When cells were treated with both Tc and IPTG, full induction of cat was reached in 24 h and maintained thereafter while in the presence of Tc alone. This suggests that regulation by Tc is fast and that the phenotypic lag may be due to slow turnover of the LacI repressor. This TTA/lacI **regulatory** system may serve as an example in which cat **expression** was used as a reporter. The data indicate that regulatory cascades regulated at multiple points can be constructed with any cloned gene in mammalian cells.

L49 ANSWER 5 OF 5 MEDLINE on STN
ACCESSION NUMBER: 95081429 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7989599
TITLE: **Regulated expression** of foreign genes in vivo after germline transfer.
AUTHOR: Passman R S; Fishman G I
CORPORATE SOURCE: Cardiology Division-Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.
CONTRACT NUMBER: 5T32HL076 (NHLBI)
HL-02391 (NHLBI)
SOURCE: Journal of clinical investigation, (1994 Dec) 94 (6) 2421-5.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950124
Last Updated on STN: 20021018
Entered Medline: 19950106

AB Tight transcriptional control of foreign genes introduced into the germline of transgenic mice would be of great experimental value in studies of gene function. To develop a system in which the spatial and

temporal expression of candidate genes implicated in cardiac development or function could be tightly controlled in vivo, we have generated transgenic mice expressing a tetracycline-controlled transactivator (**tTA**) under the control of a rat alpha myosin heavy chain **promoter** (MHC alpha-**tTA** mice), as well as mice harboring a candidate target gene implicated in the control of differentiation, Id1 (**tet**-Id1 mice). No expression of the target transgene was detected in any tissues of hemizygous **tet**-Id1 mice. Genetic crosses with MHC alpha-tTA mice resulted in transactivation of the Id1 transgene, but expression was restricted to heart, where tTA was expressed. Furthermore, transactivation of the target gene was tightly and reversibly controlled by systemic therapy with tetracycline, both in utero and postnatally. These studies demonstrate the feasibility of such a binary approach for tightly controlling the timing and extent of expression of transgenes in vivo. This approach should be generally useful for the ectopic expression of candidate genes in selected tissues during delineated developmental stages.

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L18 ANSWER 1 OF 11 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:411333 BIOSIS
DOCUMENT NUMBER: PREV200100411333
TITLE: Transgenic organisms having tetracycline-regulated transcriptional regulatory systems.
AUTHOR(S): **Bujard, Hermann** [Inventor, Reprint author]; Gossen, Manfred [Inventor]; Salfeld, Jochen G. [Inventor]; Voss, Jeffrey W. [Inventor]
CORPORATE SOURCE: Heidelberg, Germany
ASSIGNEE: BASF Aktiengesellschaft, Germany; Knoll Aktiengesellschaft, Germany
PATENT INFORMATION: US 6252136 20010626
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (June 26, 2001) Vol. 1247, No. 4. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 29 Aug 2001
Last Updated on STN: 22 Feb 2002

AB Transgenic animals carrying two transgenes, the first coding for a transactivator fusion protein comprising a **tet** repressor and a polypeptide which directly or indirectly activates in eucaryotic cells, and the second comprising a gene operably linked to a minimal **promoter** operably linked to at least one **tet** operator sequence, are disclosed. Isolated DNA molecules (e.g., targeting vectors) for integrating a polynucleotide sequence encoding a transactivator of the invention at a predetermined location within a second target DNA molecule by homologous recombination are also disclosed. Transgenic animals having the DNA molecules of the invention integrated at a predetermined location in a chromosome by homologous recombination are also encompassed by the invention. Methods to regulate the expression of a **tet** operator linked-gene of interest by administering tetracycline or a tetracycline analogue to an animal of the invention are also disclosed. The regulatory system of the invention allows for conditional inactivation or modulation of expression of a gene of interest in a host cell or animal.

L18 ANSWER 2 OF 11 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:539288 BIOSIS
DOCUMENT NUMBER: PREV200100539288
TITLE: Transgenic organisms having tetracycline-regulated transcriptional regulatory systems.
AUTHOR(S): **Bujard, Hermann** [Inventor, Reprint author]; Gossen, Manfred [Inventor]
CORPORATE SOURCE: Heidelberg, Germany
ASSIGNEE: BASF Aktiengesellschaft, Germany; Knoll Aktiengesekkscgaft, Germany
PATENT INFORMATION: US 6242667 20010605
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (June 5, 2001) Vol. 1247, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Nov 2001
Last Updated on STN: 25 Feb 2002

AB Transgenic animals carrying a transgene comprising a nucleic acid molecule encoding protein useful for regulating the expression of genes in eukaryotic cells and organisms in a highly controlled manner are disclosed. In the regulatory system of the invention, transcription of a **tet** operator-linked nucleotide sequence is stimulated by a transcriptional **activator** fusion protein composed of two polypeptides, a first polypeptide which binds to **tet** operator sequences in the presence of tetracycline operatively linked to a second polypeptide activates transcription in eukaryotic cells. In a preferred embodiment, the transgene encoding the transcriptional **activator** fusion protein is integrated at a predetermined location within the chromosome of the transgenic animal.

L18 ANSWER 3 OF 11 EMBASE

on STN
ACCESSION NUMBER: 2002062363 EMBASE
TITLE: Regulated and prolonged expression of mIFNα in immunocompetent mice mediated by a helper-dependent adenovirus vector.
AUTHOR: Aurisicchio L.; **Bujard H.**; Hillen W.; Cortese R.; Ciliberto G.; La Monica N.; Palombo F.
CORPORATE SOURCE: F. Palombo, IRBM P Angeletti, Via Pontina Km 30 600, 00040 Pomezia Rome, Italy
SOURCE: Gene Therapy, (2001) Vol. 8, No. 24, pp. 1817-1825.
Refs: 30
ISSN: 0969-7128 CODEN: GETHEC
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020301
Last Updated on STN: 20020301

AB A major goal in gene therapy is to develop efficient gene transfer protocols that allow tissue-specific, long-term and tightly regulated expression of the desired transgene. This objective is becoming more attainable through the co-evolution of gene transfer vectors and regulation systems. The ideal vector should efficiently transduce non-dividing cells with minimal toxicity, thus endowing the system with persistent transgene expression. The helper-dependent adenovirus vectors meet these requirements, as demonstrated in various studies in the literature. The most promising regulation system is the **tet-on system**, which has low basal transcriptional activity and high inducibility. To explore the regulated transgene expression in the context of a helper-dependent vector, we constructed the HD-**TET**-IFN vector, containing the mIFNα gene under the control of the tetracycline inducible transactivator rtTA2(s)-S2. Mice injected with HD-**TET**-IFN showed high levels of serum mIFNα only upon transcriptional activation. The transgene expression was reinducible to the same high level up to 3 months p.i., and the amount of expressed cytokine could be regulated by dosing doxycycline. Transcriptional activation of mIFNα induced by doxycycline resulted in prolonged survival and reduced liver damage in HD-**TET**-IFN-injected mice challenged with a lethal dose of coronavirus. Activation of antiviral genes mediated by doxycycline-dependent mIFNα expression was also observed at low HD-**TET**-IFN doses. The possibility of controlling gene expression by the combination of HD vectors and the latest **tet**-on transactivator also holds promise for studying gene function in other animal models.

L18 ANSWER 4 OF 11 MEDLINE on STN
ACCESSION NUMBER: 2001667896 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11713335
TITLE: Modulation of myosin A expression by a newly established tetracycline repressor-based inducible system in Toxoplasma gondii.
AUTHOR: Meissner M; Brecht S; **Bujard H**; Soldati D
CORPORATE SOURCE: Zentrum fur Molekulare Biologie der Universitat Heidelberg, Im Neuenheimer Feld 282, 69102 Heidelberg, Germany.
SOURCE: Nucleic acids research, (2001 Nov 15) 29 (22) E115.
Journal code: 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011120
Last Updated on STN: 20020125
Entered Medline: 20020111

AB We have developed a control system for regulating gene activation in

Toxoplasma gondii. The elements of this system are derived from the Escherichia coli tetracycline resistance **operon**, which has been widely used to tightly control gene expression in eukaryotes. The tetracycline repressor (tetR) interferes with transcription initiation while the chimeric transactivator, composed of the tetR fused to the activating domain of VP16 transcriptional factor, allows **tet** -dependent transcription. Accordingly, tetracycline derivatives such as anhydrotetracycline, which we found to be well tolerated by T.gondii, can serve as effector molecules, allowing control of gene expression in a reversible manner. As a prerequisite to functionally express the tetR in T.gondii, we used a synthetic gene with change of codon frequency. Whereas no activation of transcription was achieved using the synthetic tetracycline-controlled transactivator, tTA2(s), the TetR(s)modulates parasite transcription over a range of approximately 15-fold as measured for several reporter genes. We show here that the tetR-dependent induction of the T.gondii myosin A transgene expression drastically down-regulates the level of endogenous MyoA. This myosin is under the control of a tight feedback mechanism, which occurs at the protein level.

L18 ANSWER 5 OF 11 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000389198 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10859354
TITLE: Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity.
AUTHOR: Urlinger S; Baron U; Thellmann M; Hasan M T; **Bujard H**; Hillen W
CORPORATE SOURCE: Institut fur Mikrobiologie, Universitat Erlangen, Staudtstrasse 5, D-91058 Erlangen, Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2000 Jul 5) 97 (14) 7963-8. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000810

AB Regulatory elements that control tetracycline resistance in Escherichia coli were previously converted into highly specific transcription regulation systems that function in a wide variety of eukaryotic cells. One tetracycline repressor (TetR) mutant gave rise to rtTA, a tetracycline-controlled transactivator that requires doxycycline (Dox) for binding to **tet** operators and thus for the activation of P(**tet**) promoters. Despite the intriguing properties of rtTA, its use was limited, particularly in transgenic animals, because of its relatively inefficient inducibility by doxycycline in some organs, its instability, and its residual affinity to tetO in absence of Dox, leading to elevated background activities of the target **promoter**. To remove these limitations, we have mutagenized tTA DNA and selected in Saccharomyces cerevisiae for rtTA mutants with reduced basal activity and increased Dox sensitivity. Five new rtTAs were identified, of which two have greatly improved properties. The most promising new transactivator, rtTA2(S)-M2, functions at a 10-fold lower Dox concentration than rtTA, is more stable in eukaryotic cells, and causes no background expression in the absence of Dox. The coding sequences of the new reverse TetR mutants fused to minimal activation domains were optimized for expression in human cells and synthesized. The resulting transactivators allow stringent regulation of target genes over a range of 4 to 5 orders of magnitude in stably transfected HeLa cells. These rtTA versions combine tightness of expression control with a broad regulatory range, as previously shown for the widely applied tTA.

L18 ANSWER 6 OF 11 MEDLINE on STN
ACCESSION NUMBER: 2000203067 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10738580
TITLE: A tetracycline controlled activation/repression system with

increased potential for gene transfer into mammalian cells.
AUTHOR: Freundlieb S; Schirra-Muller C; **Bujard H**
CORPORATE SOURCE: Zentrum fur Molekulare Biologie (ZMBH), Universitat Heidelberg, Germany.. sabinef@sun0.urz.uni
SOURCE: journal of gene medicine, (1999 Jan-Feb) 1 (1) 4-12. Journal code: 9815764. ISSN: 1099-498X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000414

AB BACKGROUND: Tight control of gene activity has been achieved in cells and transgenic organisms using the **Tet** regulatory systems. Unregulated basal transcription can, however, be observed whenever integration of target genes driven by promoters responsive to tetracycline controlled transcriptional activators (tTA, rtTA) does not occur at suitable chromosomal sites. Moreover, in viral vectors containing both the tTA coding sequence and the regulated target gene, proximity of the enhancer element driving tTA/rtTA expression to the responsive unit will lead to elevated background levels. Similarly when tTA/rtTA responsive transcription units are in a non-integrated state as e.g., during transient expression, intrinsic residual transcription persists in their 'off' state, which can differ in intensity among different cell types. METHODS: To efficiently repress such background activities we generated tetracycline controlled transcriptional silencers (tTS) that bind promoters responsive for rtTA in absence of the effector doxycycline (Dox). Addition of Dox prevents binding of tTS thus relieving repression, promotes binding of rtTA and thereby switches the **promoter** from an actively repressed to an activated state. RESULTS: Of several tTS--fusions between a modified **Tet** repressor and transcriptional silencing domains--tTSKid was found to be most effective in reducing the activity of two target promoters. Ten to 200 fold repression is seen in transient expression whereas in stably transfected HeLa cells the regulatory range of the rtTA system was increased by three orders of magnitude. CONCLUSIONS: The new system appears particularly suited for the transfer of toxic genes into appropriate chromosomal sites as well as for tight regulation of genes carried by viral or episomal vectors.

L18 ANSWER 7 OF 11 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 97248689 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9092630
TITLE: Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements.
AUTHOR: Lutz R; **Bujard H**
CORPORATE SOURCE: ZMBH Zentrum fur Molekulare Biologie der Universitat Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany.
SOURCE: Nucleic acids research, (1997 Mar 15) 25 (6) 1203-10. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U66308; GENBANK-U66309; GENBANK-U66310; GENBANK-U66311; GENBANK-U66312; GENBANK-U66313
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970507
Last Updated on STN: 19990129
Entered Medline: 19970430

AB Based on parameters governing **promoter** activity and using regulatory elements of the lac, ara and **tet operon** transcription control sequences were composed which permit the regulation in Escherichia coli of several gene activities independently and quantitatively. The novel **promoter** PLtetO-1 allows the

regulation of gene expression over an up to 5000-fold range with anhydrotetracycline (aTc) whereas with IPTG and arabinose the activity of Plac/ara-1 may be controlled 1800-fold. Escherichia coli host strains which produce defined amounts of the regulatory proteins, Lac and **Tet** repressor as well as AraC from chromosomally located expression units provide highly reproducible in vivo conditions. Controlling the expression of the genes encoding luciferase, the low abundance E.coli protein DnaJ and restriction endonuclease Cfr9I not only demonstrates that high levels of expression can be achieved but also suggests that under conditions of optimal repression only around one mRNA every 3rd generation is produced. This potential of quantitative control will open up new approaches in the study of gene function in vivo, in particular with low abundance regulatory gene products. The system will also provide new opportunities for the controlled expression of heterologous genes.

L18 ANSWER 8 OF 11 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 95023899 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7937760
TITLE: Temporal control of gene expression in transgenic mice by a tetracycline-responsive promoter.
AUTHOR: Furth P A; St Onge L; Boger H; Gruss P; Gossen M; Kistner A; **Bujard H**; Hennighausen L
CORPORATE SOURCE: Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, Gottingen, Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994 Sep 27) 91 (20) 9302-6.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19990129
Entered Medline: 19941027
AB Promoters whose temporal activity can be directly manipulated in transgenic animals provide a tool for the study of gene functions in vivo. We have evaluated a tetracycline-responsive binary system for its ability to temporally control gene expression in transgenic mice. In this system, a tetracycline-controlled trans-**activator** protein (tTA), composed of the repressor of the tetracycline-resistance **operon** (**tet** from Escherichia coli transposon Tn10) and the activating domain of viral protein VP16 of herpes simplex virus, induces transcription from a minimal **promoter** (PhCMV*-1; see below) fused to seven **tet** operator sequences in the absence of tetracycline but not in its presence. Transgenic mice were generated that carried either a luciferase or a beta-galactosidase reporter gene under the control of PhCMV*-1 or a transgene containing the tTA coding sequence under the control of the human cytomegalovirus immediate early gene 1 (hCMV IE1) **promoter**/enhancer. Whereas little luciferase or beta-galactosidase activity was observed in tissues of mice carrying only the reporter genes, the presence of tTA in double-transgenic mice induced expression of the reporter genes up to several thousand-fold. This induction was abrogated to basal levels upon administration of tetracycline. These findings can be used, for example, to design dominant gain-of-function experiments in which temporal control of transgene expression is required.

L18 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 94282092 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8012406
TITLE: A chimeric transactivator allows tetracycline-responsive gene expression in whole plants.
AUTHOR: Weinmann P; Gossen M; Hillen W; **Bujard H**; Gatz C
CORPORATE SOURCE: Institut fur Genbiologische Forschung Berlin GmbH, Germany.
SOURCE: Plant journal : for cell and molecular biology, (1994 Apr) 5 (4) 559-69.
Journal code: 9207397. ISSN: 0960-7412.

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940810
Last Updated on STN: 19990129
Entered Medline: 19940728

AB The chimeric transcriptional **activator** tTA, a fusion between the Tn10 encoded **Tet** repressor and the activation domain of the Herpes simplex virion protein VP16, was stably expressed in transgenic tobacco plants. It stimulates transcription of the beta-glucuronidase (gus) gene from an artificial **promoter** consisting of 7 **tet** operators and a TATA-box. Tetracycline, which interferes with binding of tTA to operator DNA, reduces gus expression over several orders of magnitude. This stringency of regulation suggests that the system can be used to construct transgenic plants encoding a potentially lethal gene product. Furthermore, the specific and fast inactivation of tTA allows study of the stability of RNAs and proteins.

L18 ANSWER 10 OF 11 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 92302280 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1319065
TITLE: Tight control of gene expression in mammalian cells by tetracycline-responsive promoters.
AUTHOR: Gossen M; **Bujard H**
CORPORATE SOURCE: Zentrum fur Molekulare Biologie, Universitat Heidelberg, Federal Republic of Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1992 Jun 15) 89 (12) 5547-51.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920731
Last Updated on STN: 19970203
Entered Medline: 19920721

AB Control elements of the tetracycline-resistance **operon** encoded in Tn10 of Escherichia coli have been utilized to establish a highly efficient regulatory system in mammalian cells. By fusing the **tet** repressor with the activating domain of virion protein 16 of herpes simplex virus, a tetracycline-controlled transactivator (tTA) was generated that is constitutively expressed in HeLa cells. This transactivator stimulates transcription from a minimal **promoter** sequence derived from the human cytomegalovirus **promoter** IE combined with **tet** operator sequences. Upon integration of a luciferase gene controlled by a tTA-dependent **promoter** into a tTA-producing HeLa cell line, high levels of luciferase expression were monitored. These activities are sensitive to tetracycline. Depending on the concentration of the antibiotic in the culture medium (0-1 microgram/ml), the luciferase activity can be regulated over up to five orders of magnitude. Thus, the system not only allows differential control of the activity of an individual gene in mammalian cells but also is suitable for creation of "on/off" situations for such genes in a reversible way.

L18 ANSWER 11 OF 11 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 84207891 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6327267
TITLE: Transcription from efficient promoters can interfere with plasmid replication and diminish expression of plasmid specified genes.
AUTHOR: Stueber D; **Bujard H**
SOURCE: EMBO journal, (1982) 1 (11) 1399-404.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198407
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19840725

AB The copy number of plasmids containing the ColE1 replicon is affected by changes in the transcriptional activity within the plasmid if these changes lead to transcriptional readthrough into the replication region towards the **promoter** priming DNA replication. Such readthrough e.g., from the **tet** region in pBR322 not only causes overproduction of a peptide known to affect the copy number negatively but also appears to interfere negatively with the replication of the plasmid itself. The proper placement of efficient transcriptional terminators prevents such interference and permits the stable integration of strong promoters. Due to this termination effect, up to 9-fold differences in plasmid copy number were observed, depending upon the particular growth conditions. The higher copy number is of course reflected by higher yields of plasmid-specified gene products indicating the relevance of the above effects for studies of gene expression.

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L41 ANSWER 1 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:412437 BIOSIS
DOCUMENT NUMBER: PREV199800412437
TITLE: Controlling gene activities via the tetracycline regulatory systems.
AUTHOR(S): Freundlieb, Sabine; Baron, Udo; Bujard, Hermann
CORPORATE SOURCE: Zentrum Mol. Biol., Univ. Heidelberg, Heidelberg D-69120, Germany
SOURCE: Celis, J. E. [Editor]. (1998) pp. 230-238. Cell biology, Vol. 4. Second edition. print.
Publisher: Academic Press, Inc., 1250 Sixth Ave., San Diego, California 92101, USA; Academic Press Ltd., 14 Belgrave Square, 24-28 Oval Road, London NW1 70X, England, UK.
ISBN: 0-12-164729-3.
DOCUMENT TYPE: Book
Book; (Book Chapter)
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Oct 1998
Last Updated on STN: 2 Oct 1998

L41 ANSWER 2 OF 28 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998445413 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9770528
TITLE: Doxycycline control of prion protein transgene expression modulates prion disease in mice.
AUTHOR: Tremblay P; Meiner Z; Galou M; Heinrich C; Petromilli C; Lisse T; Cayetano J; Torchia M; Mobley W; Bujard H; DeArmond S J; Prusiner S B
CORPORATE SOURCE: Department of Neurology, University of California, San Francisco, CA 94143, USA.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Oct 13) 95 (21) 12580-5.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981112
AB Conversion of the cellular prion protein (PrPC) into the pathogenic isoform (PrPSc) is the fundamental event underlying transmission and pathogenesis of prion diseases. To control the expression of PrPC in transgenic (Tg) mice, we used a tetracycline controlled transactivator (**tTA**) driven by the PrP gene control elements and a **tTA** -responsive **promoter** linked to a PrP gene [Gossen, M. and Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5547-5551]. Adult Tg mice showed no deleterious effects upon repression of PrPC expression (>90%) by oral doxycycline, but the mice developed progressive ataxia at approximately 50 days after inoculation with prions unless maintained on doxycycline. Although Tg mice on doxycycline accumulated low levels of PrPSc, they showed no neurologic dysfunction, indicating that low levels of PrPSc can be tolerated. Use of the tTA system to control PrP expression allowed production of Tg mice with high levels of PrP that otherwise cause many embryonic and neonatal deaths. Measurement of PrPSc clearance in Tg mice should be possible, facilitating the development of pharmacotherapeutics.

L41 ANSWER 3 OF 28 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998252810 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9584119
TITLE: Spatial and temporal targeting of gene expression in Drosophila by means of a tetracycline-dependent transactivator system.
AUTHOR: Bello B; Resendez-Perez D; Gehring W J
CORPORATE SOURCE: Biozentrum, University of Basel, Klingelbergstrasse 70,

CH-4056 Basel, Switzerland.. bbello@nimr.mrc.ac.uk
SOURCE: Development (Cambridge, England), (1998 Jun) 125 (12) 2193-202.
Journal code: 8701744. ISSN: 0950-1991.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980811
Last Updated on STN: 19980811
Entered Medline: 19980730

AB In order to evaluate the efficiency of the tetracycline-regulated gene expression system in Drosophila, we have generated transgenic lines expressing a tetracycline-controlled transactivator protein (tTA), with specific expression patterns during embryonic and larval development. These lines were used to direct expression of a **tTA**-responsive **promoter** fused to the coding region of either the beta-galactosidase or the homeotic protein Antennapedia (ANTP), under various conditions of tetracycline treatment. We found that expression of beta-galactosidase can be efficiently inhibited in embryos and larvae with tetracycline provided in the food, and that a simple removal of the larvae from tetracycline exposure results in the induction of the enzyme in a time- and concentration-dependent manner. Similar treatments can be used to prevent the lethality associated with the ectopic expression of ANTP in embryos and, subsequently, to control the timing of expression of the homeoprotein ANTP specifically in the antennal imaginal disc. Our results show that the expression of a gene placed under the control of a tetracycline-responsive **promoter** can be tightly controlled, both spatially by the regulatory sequences driving the expression of **tTA** and temporally by tetracycline. This provides the basis of a versatile binary system for controlling gene expression in Drosophila, with an additional level of regulation as compared to the general method using the yeast transcription factor GAL4.

L41 ANSWER 4 OF 28 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1998243043 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9583685
TITLE: Evaluation of an autoregulatory tetracycline regulated system.
AUTHOR: Gallia G L; Khalili K
CORPORATE SOURCE: Center for NeuroVirology and NeuroOncology, Department of Neurology, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania 19102, USA.
SOURCE: Oncogene, (1998 Apr 9) 16 (14) 1879-84.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980529
Last Updated on STN: 19980529
Entered Medline: 19980519

AB Tetracycline controlled gene expression systems have become powerful tools in the analysis of gene function in mammalian cell culture as well as transgenic animals and plants. The original description of a tetracycline-regulated gene expression system is based on two plasmids, one of which constitutively expresses a tetracycline-controlled transactivator protein (tTA), a fusion protein between the tetracycline repressor of E. coli and the transcriptional activation domain of the VP16 protein of herpes simplex virus. The second plasmid contains the gene to be regulated by **tTA** under the control of an inducible **promoter** which consists of seven copies of the tetracycline resistance operator (tetO). Since this original description, many modifications have been described. In this report, we evaluate an autoregulatory tetracycline controlled system, in which the tTA is itself under the control of the tetO. We demonstrate that this autoregulatory tetracycline system produces adverse effects including cellular

morphologic changes, growth rate attenuation and alterations in cell cycle distribution.

L41 ANSWER 5 OF 28 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999129183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9930322
TITLE: Highly controlled gene expression using combinations of a tissue-specific promoter, recombinant adenovirus and a tetracycline-regulatable transcription factor.
AUTHOR: Ghersa P; Gobert R P; Sattonnet-Roche P; Richards C A; Merlo Pich E; Hooft van Huijsduijnen R
CORPORATE SOURCE: Serono Pharmaceutical Research Institute (previously Glaxo-Wellcome), Geneva, Switzerland.
SOURCE: Gene therapy, (1998 Sep) 5 (9) 1213-20.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990225

AB Controllable gene expression is a desirable feature both in gene therapy protocols and for the study of gene function in animals and plants. We have exploited the modular character of the tetracycline (tc)-regulatable genetic switch to show that its components can be encoded by any combination of recombinant adenovirus and/or transgenic mice. Transgenic mice were constructed that express the tc-regulatable trans-activator tTA muscle specifically. These were injected with recombinant adenovirus expressing a luciferase reporter controlled by the **tTA** -regulatable **promoter**. Virus injected into muscle, but not into a control organ (brain) resulted in luciferase activity. Conversely, injection of **tTA** producing adenovirus into mice that were transgenic for a trkB/Fc fusion protein gene under tc **promoter** control resulted in swift expression of serum trkB/Fc receptor-body. Both modes of gene induction were fully inhibited by administration of tc. We demonstrate that a careful choice of these tools allows exquisite in vivo control over transgene expression in a temporal, tc-regulatable, topical and tissue-specific manner.

L41 ANSWER 6 OF 28 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1998197330 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9536267
TITLE: Efficient transgene regulation from a single tetracycline-controlled positive feedback regulatory system.
AUTHOR: A-Mohammadi S; Hawkins R E
CORPORATE SOURCE: Centre for Protein Engineering, MRC Centre, Cambridge, UK.
SOURCE: Gene therapy, (1998 Jan) 5 (1) 76-84.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 19980422
Entered Medline: 19980415

AB Control of gene expression in eukaryotic cells is clearly important in many applications including modifications of the level of a therapeutic gene product. For effective gene delivery and regulation, the regulatory system must be contained on a single vector and it must exhibit high transgene expression on induction and low basal expression on repression. Here, we have investigated several self-contained vectors carrying both the tetracycline-controlled transactivator (tTA) and a potentially therapeutic gene in transient studies. An enhancerless positive feedback regulatory vector (pSiaIV) transcribing both **tTA** and mGM-CSF from a modified **tTA**-responsive bidirectional **promoter**

demonstrated over 200-fold gene regulation in HeLa cells. This was comparable to the degree of regulation obtained on cotransfection of vectors expressing tTA and tTA-responsive mGM-CSF. The maximal transcriptional activity of pSiaIV was comparable to that of CMV IE promoter and its basal activity as low as the leakiness of the tetracycline-responsive promoter (tRP) in several commonly used cell lines, resulting in 47- to 328-fold regulation. Furthermore, pSiaIV also showed efficient regulation in stable cells. Overall, the positive feedback regulatory system (PFRS) offers efficient gene regulation which is suitable for most applications, especially gene therapy.

L41 ANSWER 7 OF 28 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 97404737 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9261449
TITLE: A conditional self-inactivating retrovirus vector that uses a tetracycline-responsive expression system.
AUTHOR: Hwang J J; Li L; Anderson W F
CORPORATE SOURCE: Gene Therapy Laboratories, Norris Cancer Center, University of Southern California School of Medicine, Los Angeles 90033, USA.
SOURCE: Journal of virology, (1997 Sep) 71 (9) 7128-31.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970926
Last Updated on STN: 19970926
Entered Medline: 19970917
AB We developed a novel conditional self-inactivating (C-SIN) vector, TL-SN, by replacement of the enhancer-promoter of the 3' long terminal repeat of Moloney murine leukemia virus with a synthetic tetracycline operator-cytomegalovirus promoter (tetP) from the tetracycline-responsive expression system (TRES). The other component of the TRES, a chimeric transactivator (tTA), was stably incorporated into PA317 amphotropic packaging cells, thus generating the packaging cell line PA317-tTA. C-SIN amphotropic G418-resistant virus particles were generated with a titer of 2 x 10(5) CFU/ml within 2 days of transinfection of PA317-tTA cells with TL-SN ecotropic virus particles. This titer was approximately 2 log units higher than that obtained by transinfection of parental PA317 cells and was due to the high level of viral transcripts originating from the tetP **promoter** at the 5' end of the transduced vector in the presence of **tTA**. Our C-SIN vector has the potential for use in human gene therapy since it incorporates the advantages of previous SIN vectors in having weak tetP **promoter** activity (in the absence of **tTA** in target cells) while at the same time achieving high viral titers with PA317-**tTA** packaging cells.

L41 ANSWER 8 OF 28 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97370046 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9224615
TITLE: An episomal vector for stable tetracycline-regulated gene expression.
AUTHOR: Jost M; Kari C; Rodeck U
CORPORATE SOURCE: The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104, USA.
CONTRACT NUMBER: CA25874-16 (NCI)
SOURCE: Nucleic acids research, (1997 Aug 1) 25 (15) 3131-4.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19971008
Last Updated on STN: 19971008
Entered Medline: 19970925
AB The recently introduced tetracycline (Tc)-regulatable eukaryotic gene

expression system based on the Escherichia coli Tn 10 tetracycline operon has proven to be a powerful tool for controlled expression of a variety of genes in vitro as well as in vivo . Control elements of this expression system are contained in two separate plasmid vectors. The **tTA** vector encodes a transactivator protein and the tetP vector contains a responsive operator-**promoter** element (tetP) that controls gene expression depending on **tTA** binding. Establishment of cell lines expressing a gene of interest under tetP control requires two subsequent rounds of transfection and clonal selection after each transfection. Here we describe a modification of this system in which the tetP element is placed in an episomal EBNA-based plasmid that can be stably maintained in primate but not in rodent cells. Using HeLa and human melanoma cells, we show that upon transient or stable transfection a reporter gene is expressed in a Tc-regulated manner similar to the original system. Thus, this expression system combines the advantages of episomal vectors, such as high efficiency of transfection and time-efficient selection of mass cultures, with tight control of gene expression provided by the Tc-regulatable system.

L41 ANSWER 9 OF 28 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 97440934 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9296393
TITLE: Tetracycline regulated expression of vimentin in fibroblasts derived from vimentin null mice.
AUTHOR: Holwell T A; Schweitzer S C; Evans R M
CORPORATE SOURCE: Department of Pathology, University of Colorado Health Sciences Center, Denver 80262, USA.
CONTRACT NUMBER: HL51850 (NHLBI)
SOURCE: Journal of cell science, (1997 Aug) 110 (Pt 16) 1947-56. Journal code: 0052457. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971024
Last Updated on STN: 19971024
Entered Medline: 19971016

AB Fibroblast cell lines were derived from vim-/- mice that express a mouse vimentin transgene in a tetracycline regulatable manner. Vimentin null mouse primary embryo fibroblasts were transformed with SV-40 early genes and vim- cell lines were isolated. A vim- cell line was then serially transfected with an expression plasmid encoding the tetracycline regulatable transactivator (**tTA**) and a mouse vimentin cDNA expression plasmid under the regulation of Escherichia coli tet operator and minimal CMV **promoter** sequences. Two stable cell lines were obtained that contained little or no vimentin in the presence of low concentrations of tetracycline but rapidly expressed abundant vimentin filaments after removal of tetracycline. The vimentin content of one cell line was similar to that of control vim+/+ fibroblasts. The level of transgene expression could be regulated by the concentration of tetracycline in a dose dependent fashion. Induction of vimentin expression in these cells did not observably affect cell growth, the distribution of microfilaments or microtubules, or the shape of the nucleus. Enucleation studies indicated that while disassembly of microfilaments significantly increased the sensitivity of the cells to enucleation, the presence or absence of vimentin had no detectable effect on the degree of enucleation with increasing sedimentation force. Monolayer wounding experiments demonstrated that vimentin expression did not alter the mobility of polarized cells at the edge of the wound. Experiments to more directly test the effect of vimentin expression on the capacity of these fibroblasts to survive mechanical trauma indicated that vimentin expression had no obvious effect on the survival of suspension cells subjected to nitrogen cavitation or the fraction of cells that survived the mechanical scraping of monolayer culture. These studies indicate that vimentin expression in a single population of cells does not have an obvious effect on cytoplasmic organization and provides a useful system to study the effects of IFs on the capacity of individual cells to resist mechanical injury.

L41 ANSWER 10 OF 28 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1998010150 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9349437
TITLE: Delay in resumption of the activity of tetracycline-regulatable promoter following removal of tetracycline analogues.
AUTHOR: A-Mohammadi S; Alvarez-Vallina L; Ashworth L J; Hawkins R E
CORPORATE SOURCE: Centre for Protein Engineering, MRC Centre, Cambridge, UK.
SOURCE: Gene therapy, (1997 Sep) 4 (9) 993-7.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971120

AB The tetracycline-regulatable system (TRS) has become a widely adopted tool for modification of gene expression and analysis of gene function in mammalian cells, plants and transgenic animals. We have studied the potential application of the TRS in gene therapy, using a single vector containing both the tetracycline-controlled transactivator (**tTA**) and the **tTA**-responsive **promoter** (tRP) transcribing mouse GM-CSF. Stable 293 cells established using this vector were used to study the kinetics of the TRS in response to various tetracycline analogues. Dose-response studies show that doxycycline is the most potent-analogue in abolishing tTA activity. Kinetic studies indicate that, at 1,000 ng/ml, all the analogues have similar efficiencies in down-regulating the system in given time. In contrast, following the removal of the analogues, there is a temporal, dose-dependent delay in resumption of the tRP activity. The time taken for resumption of near-optimal tRP activity is approximately 48 h for tetracycline, 144 h for anhydrotetracycline, 192 h for minocycline and 216 h for doxycycline when cells were pretreated with 1000 ng/ml of these antibiotics. This property of the analogues can be employed in planning a desired course of transgene regulation.

L41 ANSWER 11 OF 28 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 97377991 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9234672
TITLE: A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in Saccharomyces cerevisiae.
AUTHOR: Gari E; Piedrafita L; Aldea M; Herrero E
CORPORATE SOURCE: Departament de Ciencies Mediques Basiques, Facultat de Medicina, Universitat de Lleida, Spain.
SOURCE: Yeast (Chichester, England), (1997 Jul) 13 (9) 837-48.
Journal code: 8607637. ISSN: 0749-503X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19970929

AB A set of Saccharomyces cerevisiae expression vectors has been developed in which transcription is driven by a hybrid tetO-CYC1 **promoter** through the action of a tetR-VP16 (**tTA**) activator. Expression from the promoter is regulated by tetracycline or derivatives. Various modalities of promoter and activator are used in order to achieve different levels of maximal expression. In the presence of antibiotic in the growth medium at concentrations that do not affect cell growth, expression from the tetO promoter is negligible, and upon antibiotic removal induction ratios of up to 1000-fold are observed with a lacZ reporter system. With the strongest system, overexpression levels comparable with those observed with GAL1-driven promoters are reached.

For each particular **promoter/tTA** combination, expression can be modulated by changing the tetracycline concentration in the growth medium. These vectors may be useful for the study of the function of essential genes in yeast, as well as for phenotypic analysis of genes in overexpression conditions, without restrictions imposed by growth medium composition.

L41 ANSWER 12 OF 28 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1998074611 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9413131
TITLE: Autoregulated multicistronic expression vectors provide one-step cloning of regulated product gene expression in mammalian cells.
AUTHOR: Fussenegger M; Moser S; Mazur X; Bailey J E
CORPORATE SOURCE: Institute of Biotechnology, ETH Zurich, Switzerland.
SOURCE: Biotechnology progress, (1997 Nov-Dec) 13 (6) 733-40. Journal code: 8506292. ISSN: 8756-7938.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980227

AB Regulated expression of a cloned gene often provides much higher final expression of the gene product. Also, regulated expression of an activity can enable optional metabolic engineering and simplify functional genomic research. We constructed di-, tri-, and quattrocistronic mammalian expression vectors which allow the simultaneous, coordinated, and adjustable expression of up to two product genes. A single, tetracycline-regulatable **promoter**, PhCMV*-1, drives high-level expression of a multicistronic expression unit, containing the product gene(s), the gene for tetracycline-responsive transactivator (**tTA**), and, in the case of pQuattro-**tTA**, also the neomycin resistance gene. This autoregulatory genetic configuration retains a very low basal transcription activity in the presence of tetracycline, thereby reducing or eliminating possible toxic effects of tTA expression. However, upon withdrawal of tetracycline, a positive feedback regulation loop is activated which leads to higher levels of tTA expression and consequently also to higher expression levels of all other cistrons encoded on the multicistronic expression unit. Since such multicistronic expression vectors combine all genetic elements necessary for high-level expression as well as regulation in a single multicistronic expression unit, they alleviate limitations of previously reported tetracycline-regulatable vector systems and allow straightforward, one-step genetic engineering of eucaryotic cells to give an adjustable phenotype under strict control of the external stimulus, here tetracycline. Because the expression vectors described here were used for the expression for several heterologous product genes such as the green fluorescent protein and the tumor suppressor gene p21 in several cell lines (CHO-K1, BHK-21, and HeLa), we expect these multicistronic, positive feedback regulation vectors to function in a wide variety of eucaryotic cells and to be useful for basic as well as for applied research applications. Other vectors based upon the same autoregulation and multicistronic expression concepts can be constructed using other regulator gene-regulated promoter elements.

L41 ANSWER 13 OF 28 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 97168989 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9016796
TITLE: Adenovirus-mediated inducible gene expression through tetracycline-controllable transactivator with nuclear localization signal.
AUTHOR: Yoshida Y; Hamada H
CORPORATE SOURCE: Department of Molecular Biotherapy Research, Cancer Chemotherapy Center, Cancer Institute, Toshima-ku, Tokyo, Japan.
SOURCE: Biochemical and biophysical research communications, (1997

Jan 13) 230 (2) 426-30.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 19970313
Entered Medline: 19970305

AB Tetracycline-controllable expression vectors are widely used for inducible expression in mammalian cells. The limitation of this system is the difficulty in expressing high levels of the chimeric transactivator tTA. In this study, we demonstrate a utility of recombinant adenoviruses for the tetracycline-controllable expression system. Unexpectedly, the original **tTA** transactivator did not show sufficient regulation of the reporter gene expression driven by the tetracycline-responsive **promoter** (Tet). By adding the nuclear localization signal on the tTA transactivator (NtTA), we achieved tight regulation and high-level induction of the reporter gene expression. The NtTA driven by various promoters demonstrated strict tetracycline controllability at 1 microg/ml of tetracycline and above. The methodology for adenovirus-mediated inducible gene expression has wide applicability. Controllable expression of cytotoxic viral proteins will be applicable for antiviral vaccine productions and pseudotype viral vector generations.

L41 ANSWER 14 OF 28 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 97169880 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9017423
TITLE: Modulation of erythropoietin delivery from engineered muscles in mice.
AUTHOR: Bohl D; Heard J M
CORPORATE SOURCE: Laboratoire Retrovirus et Transfert Genetique, Institut Pasteur, Paris, France.
SOURCE: Human gene therapy, (1997 Jan 20) 8 (2) 195-204.
Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970612
Last Updated on STN: 19970612
Entered Medline: 19970603

AB In most relevant diseases, the permanent systemic delivery of a therapeutic protein from engineered cells might be proposed only if secretion levels can be regulated. The tetracycline resistance operon of Escherichia coli provides a transcriptional regulatory system effective in mammalian cells, which could be used for that purpose. A chimeric transactivator (**tTA**) consisting of the tetracycline repressor fused to the transactivation domain of the herpes simplex virus VP16 protein stimulates transcription by binding a minimal cytomegalovirus (CMV) **promoter** containing repeats of the tetracycline operator (tetO-CMV). Binding is abolished by tetracycline, thus impairing promoter activation. We have transduced C2.7 myoblasts with two U3-deleted retroviral vectors containing these regulatory elements. The tetP-Epo vector expressed the murine erythropoietin (Epo) cDNA under the control of the tetO-CMV promoter. The D-De-**tTA** vector expressed **tTA** under the control of the muscle-specific human desmin enhancer-**promoter**. Northern blot analysis showed background Epo mRNA expression in myoblasts. Myotubes differentiation induced tTA expression, leading to a 28-fold increase of Epo mRNAs, which was suppressed by tetracycline. Basal Epo secretion in myoblasts increased 23- to 41-fold during the formation of multinucleated myotubes, and turned back close to myoblast level when tetracycline was added. Myoblasts transduced with both vectors and treated with mitomycin with the aim to prevent tumor formation were engrafted in skeletal muscles of syngeneic C3H mice. Hematocrit levels were significantly higher in animals bearing cells transduced with both vectors than in control animals grafted with

cells transduced with the Epo vector only. This difference was abolished when tetracycline was given to mice. These data indicate that the tetracycline regulatory elements can modulate transcription in the context of retroviral vector genomes, and that this system can be used to control the in vivo delivery of a therapeutic protein from genetically modified muscles.

L41 ANSWER 15 OF 28 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 97148030 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8994662
TITLE: Use of a dicistronic expression cassette encoding the green fluorescent protein for the screening and selection of cells expressing inducible gene products.
AUTHOR: Mosser D D; Caron A W; Bourget L; Jolicoeur P; Massie B
CORPORATE SOURCE: National Research Council of Canada, Biotechnology Research Institute, Montreal, QC, Canada.. dick.mosser@nrc.ca
SOURCE: BioTechniques, (1997 Jan) 22 (1) 150-4, 156, 158-61.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970407
Last Updated on STN: 19970407
Entered Medline: 19970325

AB To facilitate the screening and selection of cells expressing inducible gene products, we have constructed a plasmid that, by the inclusion of a viral internal ribosome entry site, permits the synthesis of a dicistronic mRNA encoding both a gene of interest and the gene encoding the green fluorescent protein (GFP) from the jellyfish Aequorea victoria. This greatly simplifies the task of clone selection, since GFP fluorescence can be visualized non-obtrusively in live cells with a standard fluorescence microscope. We have applied this method to the tetracycline-regulated expression system in which the expression of a target gene, placed under the control of a **promoter** containing the tetracycline operator sequence (tetO), can be induced by a tetracycline-regulated trans-activator protein (**tTA**). Binding of the tTA to the tetO is inhibited in the presence of tetracycline. Optimal results with this system require two sequential rounds of transfection and screening. Obtaining a cell line expressing high levels of functional tTA is greatly simplified by transiently transfecting a plasmid encoding GFP into a pool of cells that has first been transfected with a tTA-expressor construct and selecting GFP-positive cells using a fluorescence-activated cell sorter. In the second step, the tTA cell line can then be stably transfected with a dicistronic expressor-GFP cassette. This method eliminates the task of characterizing cell lines by the standard method of examining levels of the exogenously expressed protein in cell extracts of individual clones.

L41 ANSWER 16 OF 28 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 97345779 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9202271
TITLE: Regulated expression of the diphtheria toxin A gene in human glioma cells using prokaryotic transcriptional control elements.
AUTHOR: Paulus W; Baur I; Oberer D M; Breakefield X O; Reeves S A
CORPORATE SOURCE: Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, USA.
CONTRACT NUMBER: NS24279 (NINDS)
SOURCE: Journal of neurosurgery, (1997 Jul) 87 (1) 89-95.
Journal code: 0253357. ISSN: 0022-3085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805

Entered Medline: 19970722

AB Because accurate regulation of toxin gene expression is critical for safe and effective gene therapy applications, the authors have examined the regulation of diphtheria toxin A (DTA) fragment expression in human glioma cell lines using two transcriptional control systems derived from Escherichia coli: the tetracycline (Tet) system and the lactose (Lac) system. The Tet system includes a tetracycline-controlled transactivator (**tTA**), a **tTA**-responsive minimum human cytomegalovirus (hCMV) **promoter** controlling the expression of the DTA gene, and tetracycline as an allosteric inhibitor. The Lac system includes the lac repressor (lacR), a lacR-regulated Rous sarcoma virus-long terminal repeat (RSV-LTR) promoter controlling the expression of the DTA gene, and isopropyl-thio-beta-D-galactoside (IPTG) as an allosteric inducer. Expression plasmids encoding either tTA or lacR were transfected into U-87MG and U-343MG glioma cells along with the responsive DTA plasmid. Cell killing was monitored by the ability of the toxin to abolish protein synthesis and was quantitated using a luciferase reporter gene. In the Tet system, tumor cell killing could be regulated by tetracycline up to 120-fold. In contrast, only a twofold IPTG-dependent regulation was obtained using the Lac system because of an incomplete repression of DTA expression in the uninduced state. Replacement of the RSV-LTR promoter with the heavy metal-inducible mouse metallothionein-1 promoter in the lacR-responsive unit, as well as the generation of a clonal glioma cell line expressing lacR, did not significantly enhance regulation of DTA in the Lac system. In conclusion, this study demonstrates that the Tet system is of potential use in gene therapy applications in which regulated expression of a therapeutic gene is an important issue.

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ACCESSION NUMBER: 1998:132433 BIOSIS
DOCUMENT NUMBER: PREV199800132433
TITLE: Efficient and inducible production of human interleukin 6 in Chinese hamster ovary cells using a novel expression system.
AUTHOR(S): Zhang, Yingpei; Katakura, Yoshinori; Ohashi, Hideya; Shirahata, Sanetaka [Reprint author]
CORPORATE SOURCE: Lab. Cell. Regulation Technol., Grad. Sch. Genetic Resources Technol., Kyushu Univ., 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-81, Japan
SOURCE: Cytotechnology, (1997) Vol. 25, No. 1-3, pp. 53-60. print. ISSN: 0920-9069.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Mar 1998
Last Updated on STN: 20 Mar 1998

AB High level and inducible production of human interleukin 6 (hIL-6) was achieved using a novel expression system in Chinese hamster ovary (CHO) cells. In this system, the transcription of hIL-6 gene under the control of PhCMV* -1 **promoter** composed of tetracycline operator sequences and a minimal **promoter** is activated by a chimeric transactivator (**tTA**) composed of tetracycline repressor and transactivating domain of VP16 protein of herpes simplex virus. The transcription of **tTA** gene, which is also under the control of PhCMV*-1 **promoter**, is activated by itself via a positive feedback cycle. The expression of both genes is further enhanced by potentiating the VP16 transactivating domain of tTA transactivator with pX protein of hepatitis B virus. In the presence of tetracycline, the **tTA** transactivators can not bind to PhCMV*-1 **promoter**, therefore, the expression of hIL-6 and **tTA** gene is suppressed, and the pX will not activate basal transcription. In the absence of tetracycline, **tTA** transactivators bind to PhCMV* -I **promoter** and activate efficient transcription of hIL-6 and **tTA** gene, and the transcription is further enhanced by pX via VP16 transactivating domain. Using this strategy, we isolated a clone (UX1) producing hIL-6 at a rate about 1425 ng/106 cells/day. Furthermore, the hIL-6 production is stringently regulated by tetracycline. This results suggested a novel strategy to establish highly efficient, inducible and cell type independent recombinant protein production system by using an

artificial promoter to recruit transactivators and coactivators which can synergistically activate transcription.

L41 ANSWER 18 OF 28 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 96293535 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8693003
TITLE: Creation of a reversible on/off system for site-specific in vivo control of exogenous gene activity in the renal glomerulus.
AUTHOR: Kitamura M
CORPORATE SOURCE: Glomerular Engineering Unit, Department of Medicne, University College London Medical School, United Kingdom.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1996 Jul 9) 93 (14) 7387-91. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19960911
Entered Medline: 19960829

AB Using genetically engineered glomerular mesangial cells, an in vivo gene transfer approach was developed that specifically targets the renal glomerulus. By combining this system with a tetracycline (Tc)-responsive promoter, the present study aimed to create a reversible on/off system for site-specific in vivo control of exogenous gene activity within the glomerulus. In the Tc regulatory system, a Tc-controlled transactivator (**tTA**) encoded by a regulator plasmid induces target gene transcription by binding to a **tTA**-responsive **promoter** located in a response plasmid. Tc inhibits this tTA-dependent transactivation via its affinity for tTA. In double-transfected cells, therefore, the activity of a transgene can be controlled by Tc. Cultured rat mesangial cells were cotransfected with a regulator plasmid and a response plasmid that introduces a beta-galactosidase gene. In vitro, stable double-transfectant MtTAG cells exhibited no beta-galactosidase activity in the presence of Tc. However, following withdrawal of Tc from culture media, expression of beta-galactosidase was induced within 24 h. When Tc was again added, the expression was rapidly resuppressed. Low concentrations of Tc were sufficient to maintain the silent state of **tTA**-dependent **promoter**. MtTAG cells were then transferred into the rat glomeruli via renal artery injection. In the isolated chimeric glomeruli, expression of beta-galactosidase was induced ex vivo in the absence of Tc, whereas it was repressed in its presence. When Tc-pretreated MtTAG cells were transferred into the glomeruli of untreated rats, beta-galactosidase expression was induced in vivo within 3 days. Oral administration of Tc dramatically suppressed this induction. These data demonstrate the feasibility of using mesangial cell vectors combined with the Tc regulatory system for site-specific in vivo control of exogenous gene expression in the glomerulus.

L41 ANSWER 19 OF 28 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 96323122 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8709228
TITLE: Inducible gene expression by retrovirus-mediated transfer of a modified tetracycline-regulated system.
AUTHOR: Iida A; Chen S T; Friedmann T; Yee J K
CORPORATE SOURCE: Department of Pediatrics, City of Hope National Medical Center, Duarte, California 91010-3000, USA.
CONTRACT NUMBER: HD20034 (NICHD)
SOURCE: Journal of virology, (1996 Sep) 70 (9) 6054-9. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919

Last Updated on STN: 19980206
Entered Medline: 19960910

AB The ability to regulate gene expression via exogenous stimuli will facilitate the study of gene functions in mammalian cells. In the present study, we modified the tetracycline-controlled inducible system by the addition of the ligand-binding domain of the estrogen receptor to the carboxy terminus of the tTA transactivator. A single retroviral vector can transduce both the transactivator gene and the gene of interest controlled by the **tTA**-inducible **promoter** into mammalian cells. We show that cell lines expressing the transactivator can readily be established and that expression of the gene of interest depends on the removal of tetracycline and the addition of estrogen. By using this system, cell lines with inducible expression of the G protein of vesicular stomatitis virus, a potentially toxic gene product, were established. The combination of a powerful inducible system and retrovirus-mediated gene transfer can not only be used to study gene function but may also be applied in the future to clinical trials in human gene therapy.

L41 ANSWER 20 OF 28 MEDLINE on STN DUPLICATE 18
ACCESSION NUMBER: 97128937 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8973477
TITLE: Diffuse brain invasion of glioma cells requires beta 1 integrins.
AUTHOR: Paulus W; Baur I; Beutler A S; Reeves S A
CORPORATE SOURCE: Division of Neuropathology, University of Erlangen Medical School, Germany.
SOURCE: Laboratory investigation; a journal of technical methods and pathology, (1996 Dec) 75 (6) 819-26.
Journal code: 0376617. ISSN: 0023-6837.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970122

AB Diffuse invasion of brain tissue by single tumor cells is a characteristic feature of gliomas and a major reason why these tumors cannot be completely resected. The molecular basis of brain invasion is poorly understood. We regulated the expression of beta 1 integrins, the major group of extracellular matrix receptors, in astrocytic tumor cells by using a tetracycline-dependent transcription control system. Rat C6 glioma cells were stably transfected with (a) the tetracycline-controlled transactivator (**tTA**) gene, (b) antisense beta 1 cDNA under the control of a **tTA**/tetracycline-responsive **promoter**, and (c) the beta-galactosidase (lacZ) gene for histochemical identification. In one clone, C6TL beta, beta 1 protein levels were unaffected in the presence of tetracycline, but they were reduced by 60% in the absence of tetracycline because of production of antisense mRNA. C6TL beta cells were transplanted into the striatum of nude mice. After 14 days in the presence of tetracycline in the drinking water, tumors showed diffuse brain invasion, mainly along vascular basement membranes. In the absence of tetracycline, however, tumor cells were compact and generally well delineated from the surrounding brain tissue. These data, ie, blocking of brain invasion by antisense beta 1 mRNA, either because of disturbed interaction of beta 1 with brain extracellular matrix components or interference with beta 1-dependent signaling pathways, strongly suggest that beta 1 integrins are required for diffuse brain invasion of gliomas.

L41 ANSWER 21 OF 28 MEDLINE on STN DUPLICATE 19
ACCESSION NUMBER: 96269409 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8682308
TITLE: Controlled gene expression in mammalian cells via a regulatory cascade involving the tetracycline transactivator and lac repressor.
AUTHOR: Aubrecht J; Manivasakam P; Schiestl R H
CORPORATE SOURCE: Department of Molecular and Cellular Toxicology, Harvard

School of Public Health, Boston, MA 02115, USA.
SOURCE: Gene, (1996 Jun 26) 172 (2) 227-31.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960828
Last Updated on STN: 19960828
Entered Medline: 19960822

AB Regulatory cascades or regulons control pathways at multiple points or multiple genes by one initial signal. In this paper, we describe the construction of an artificial regulatory cascade in CHO cells, which responded to various concentrations of tetracycline (Tc) and/or IPTG. The system consists of the constitutively produced transactivator (**TTA**) of the Tc operon (tet), which induced the expression of a lacI gene controlled by tet operator (tetO) and upstream CMV **promoter** (p*CMV) sequences. LacI repressed the activity of a cat gene by binding to lacO sites in its upstream RSV promoter (pRSV) region. However, this repression could be alleviated by exposure to Tc or IPTG, which inhibited the binding activities of TTA and LacI, respectively. Hence, treatment with either Tc or IPTG led to a tenfold increase in CAT activity. After the withdrawal of the inducer, cat expression reverted to basal levels. Regulation by Tc showed a phenotypic lag, and full induction was reached after 192 h, whereas IPTG addition led to full induction within 24 h. When cells were treated with both Tc and IPTG, full induction of cat was reached in 24 h and maintained thereafter while in the presence of Tc alone. This suggests that regulation by Tc is fast and that the phenotypic lag may be due to slow turnover of the LacI repressor. This TTA/lacI regulatory system may serve as an example in which cat expression was used as a reporter. The data indicate that regulatory cascades regulated at multiple points can be constructed with any cloned gene in mammalian cells.

L41 ANSWER 22 OF 28 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 96266426 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8661425
TITLE: 293 cell lines that inducibly express high levels of adenovirus type 5 precursor terminal protein.
AUTHOR: Langer S J; Schaack J
CORPORATE SOURCE: Department of Microbiology, University of Colorado Health Sciences Center, Denver 80262, USA.
CONTRACT NUMBER: GM42555 (NIGMS)
SOURCE: Virology, (1996 Jul 1) 221 (1) 172-9.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960822
Last Updated on STN: 19960822
Entered Medline: 19960814

AB 293 cell lines that inducibly express high levels of adenovirus type 5 precursor terminal protein (pTP) under the control of a tetracycline-dependent promoter were constructed. To construct the cell lines expressing pTP, 293 cells were stably transfected with a plasmid encoding the tetracycline repressor/VP16 transactivator protein (tTA) using selection with hygromycin. Cell lines that expressed high levels of **tTA** activity were then stably transfected with plasmids in which pTP expression is directed by the **tTA**-dependent **promoter** from either a cDNA or a modified genomic construct using selection with G418. Cell lines that expressed high, inducible levels of pTP efficiently complemented a temperature-sensitive pTP mutant virus for growth and plaque formation at the nonpermissive temperature.

L41 ANSWER 23 OF 28 MEDLINE on STN DUPLICATE 21
ACCESSION NUMBER: 95327679 MEDLINE

DOCUMENT NUMBER: PubMed ID: 7604026
TITLE: A modified tetracycline-regulated system provides autoregulatory, inducible gene expression in cultured cells and transgenic mice.
AUTHOR: Shockett P; Difilippantonio M; Hellman N; Schatz D G
CORPORATE SOURCE: Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06520-8011, USA.
CONTRACT NUMBER: T32-AI07019 (NIAID)
T32-HD70149 (NICHD)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1995 Jul 3) 92 (14) 6522-6. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950822
Last Updated on STN: 19950822
Entered Medline: 19950810

AB A system for tetracycline-regulated inducible gene expression was described recently which relies on constitutive expression of a tetracycline-controlled transactivator (tTA) fusion protein combining the tetracycline repressor and the transcriptional activation domain of VP16 [Gossen, M. & Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5547-5551]. This system yielded only low levels of transactivator protein, probably because tTA is toxic. To avoid this difficulty, we placed the **tTA** gene under the control of the inducible **promoter** to which **tTA** binds, making expression of **tTA** itself inducible and autoregulatory. When used to drive expression of the recombination activating genes 1 and 2 (RAG-1 and RAG-2), the autoregulatory system yielded both substantially higher levels of variable (diversity) joining [V(D)J] recombination activity (70-fold on average) and inducible expression in a much larger fraction of transfected cells (autoregulatory, 90%, vs. constitutive, 18%). In addition, this system allowed the creation of transgenic mice in which expression of a luciferase transgene was inducible tens to hundreds of times the basal levels in most tissues examined. Induced levels of expression were highest in thymus and lung and appear to be substantially higher than in previously reported inducible luciferase transgenic mice created with the constitutive system. With the modified system, inducible transactivator mRNA and protein were easily detected in cell lines by RNA and Western blotting, and transactivator mRNA was detected by RNA blotting in some tissues of transgenic mice. This autoregulatory system represents an improved strategy for tetracycline-regulated gene expression both in cultured cells and in transgenic animals.

L41 ANSWER 24 OF 28 MEDLINE on STN DUPLICATE 22
ACCESSION NUMBER: 95191037 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7884907
TITLE: Tetracycline repressor-regulated gene repression in recombinant human cytomegalovirus.
AUTHOR: Kim H J; Gatz C; Hillen W; Jones T R
CORPORATE SOURCE: Molecular Biology Section, American Cyanamid Co., Pearl River, New York 10965.
SOURCE: Journal of virology, (1995 Apr) 69 (4) 2565-73. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 19950425
Entered Medline: 19950410

AB The tetracycline repressor (TetR)-regulated gene expression system from Escherichia coli was used to control gene expression in recombinant human cytomegalovirus (HCMV). To adapt the TetR system in HCMV, derivatives of the viral US11 (early) gene promoter, which controls the

beta-glucuronidase reporter gene, were constructed by systematic insertion of the tetracycline operator (tetO) sequences. Gene expression from constructs containing two or three appropriately placed tetO sequences adjacent to the TATA box were efficiently repressed by a TetR-VP16 fusion protein (tTA) in a transient expression system. Efficient repression (50- to 120-fold) also occurred in **tTA**-expressing stably transfected human cells which were infected with recombinant HCMV containing a US11 **promoter** surrounded by three tetO sequences. The tTA-mediated gene repression was relieved in the presence of 1 microgram of tetracycline per ml. The results of this study are significant in three respects. (i) This is the first demonstration that a TetR-derived protein can be used to efficiently repress gene expression in a mammalian system. (ii) Efficient repression was dependent on the presence of the transcriptional activation domain from the herpes simplex virus type 1 VP16 protein. (iii) The ability to regulate gene expression in a controlled fashion in order to elucidate viral gene function is an important development in the HCMV field. The tTA-mediated gene repression system may be extremely useful for creating host-range mutants in essential genes in order to study their role in the HCMV replicative cycle, a system that is otherwise exceedingly difficult to genetically dissect.

L41 ANSWER 25 OF 28 MEDLINE on STN DUPLICATE 23
ACCESSION NUMBER: 96340957 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8749716
TITLE: Conditional gene expression in secretory tissues and skin of transgenic mice using the MMTV-LTR and the tetracycline responsive system.
AUTHOR: Hennighausen L; Wall R J; Tillmann U; Li M; Furth P A
CORPORATE SOURCE: Laboratory of Biochemistry and Metabolism, National Institute of Diabetes, USA.. lotharh@amb.niddk.nih.gov
SOURCE: Journal of cellular biochemistry, (1995 Dec) 59 (4) 463-72. Journal code: 8205768. ISSN: 0730-2312.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961106
Last Updated on STN: 19970203
Entered Medline: 19961023

AB Molecular mechanisms of development and disease can be studied in transgenic animals. Controlling the spatial and temporal expression patterns of transgenes, however, is a prerequisite for the elucidation of gene function in the whole organism. Previously we reported that mice carrying a tetR/VP16 hybrid gene (**tTA**), under the control of the human cytomegalovirus immediate early 1 (HCMV-IE1) gene **promoter** , can be used to temporally activate the expression of transgenes under the control of a **promoter** containing tetop sequences. We now show that the MMTV-LTR can be used to target expression of tTA to the epithelial cells of secretory organs and skin in transgenic mice. Notably, nearly uniform expression of a tetop-lacZ transgene was found in seminal vesicle, salivary gland, and Leydig cells of mice carrying also the MMTV-tTA transgene. More heterogeneous patterns of gene expression were observed in mammary epithelial cells and basal cells of the epidermis. Different MMTV-tTA lines had comparable tissue expression patterns. Transcriptional activation mediated by tTA was up to several hundredfold, and it was abrogated after the administration of tetracycline. The MMTV-tTA mice established in this work will be useful for experiments examining the roles of biological factors at defined developmental stages in the epithelial cells of salivary gland, seminal vesicle, mammary gland, and skin and the Leydig cells of testes. In addition, in combination with the CRE/lox recombination system, these mice wil be useful to achieve gene deletions at defined time points in these organs.

L41 ANSWER 26 OF 28 MEDLINE on STN DUPLICATE 24
ACCESSION NUMBER: 95023899 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7937760

TITLE: Temporal control of gene expression in transgenic mice by a tetracycline-responsive promoter.
AUTHOR: Furth P A; St Onge L; Boger H; Gruss P; Gossen M; Kistner A; Bujard H; Hennighausen L
CORPORATE SOURCE: Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, Gottingen, Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994 Sep 27) 91 (20) 9302-6.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19990129
Entered Medline: 19941027

AB Promoters whose temporal activity can be directly manipulated in transgenic animals provide a tool for the study of gene functions in vivo. We have evaluated a tetracycline-responsive binary system for its ability to temporally control gene expression in transgenic mice. In this system, a tetracycline-controlled trans-activator protein (**tTA**), composed of the repressor of the tetracycline-resistance operon (tet from Escherichia coli transposon Tn10) and the activating domain of viral protein VP16 of herpes simplex virus, induces transcription from a minimal **promoter** (PhCMV*-1; see below) fused to seven tet operator sequences in the absence of tetracycline but not in its presence. Transgenic mice were generated that carried either a luciferase or a beta-galactosidase reporter gene under the control of PhCMV*-1 or a transgene containing the **tTA** coding sequence under the control of the human cytomegalovirus immediate early gene 1 (hCMV IE1) **promoter**/enhancer. Whereas little luciferase or beta-galactosidase activity was observed in tissues of mice carrying only the reporter genes, the presence of tTA in double-transgenic mice induced expression of the reporter genes up to several thousand-fold. This induction was abrogated to basal levels upon administration of tetracycline. These findings can be used, for example, to design dominant gain-of-function experiments in which temporal control of transgene expression is required.

L41 ANSWER 27 OF 28 MEDLINE on STN DUPLICATE 25
ACCESSION NUMBER: 95081429 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7989599
TITLE: Regulated expression of foreign genes in vivo after germline transfer.
AUTHOR: Passman R S; Fishman G I
CORPORATE SOURCE: Cardiology Division-Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.
CONTRACT NUMBER: 5T32HL076 (NHLBI)
HL-02391 (NHLBI)
SOURCE: Journal of clinical investigation, (1994 Dec) 94 (6) 2421-5.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950124
Last Updated on STN: 20021018
Entered Medline: 19950106

AB Tight transcriptional control of foreign genes introduced into the germline of transgenic mice would be of great experimental value in studies of gene function. To develop a system in which the spatial and temporal expression of candidate genes implicated in cardiac development or function could be tightly controlled in vivo, we have generated transgenic mice expressing a tetracycline-controlled transactivator (**tTA**) under the control of a rat alpha myosin heavy chain **promoter** (MHC alpha-**tTA** mice), as well as mice harboring

a candidate target gene implicated in the control of differentiation, Id1 (tet-Id1 mice). No expression of the target transgene was detected in any tissues of hemizygous tet-Id1 mice. Genetic crosses with MHC alpha-tTA mice resulted in transactivation of the Id1 transgene, but expression was restricted to heart, where tTA was expressed. Furthermore, transactivation of the target gene was tightly and reversibly controlled by systemic therapy with tetracycline, both in utero and postnatally. These studies demonstrate the feasibility of such a binary approach for tightly controlling the timing and extent of expression of transgenes in vivo. This approach should be generally useful for the ectopic expression of candidate genes in selected tissues during delineated developmental stages.

L41 ANSWER 28 OF 28 MEDLINE on STN DUPLICATE 26
ACCESSION NUMBER: 92302280 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1319065
TITLE: Tight control of gene expression in mammalian cells by tetracycline-responsive promoters.
AUTHOR: Gossen M; Bujard H
CORPORATE SOURCE: Zentrum fur Molekulare Biologie, Universitat Heidelberg, Federal Republic of Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1992 Jun 15) 89 (12) 5547-51. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920731
Last Updated on STN: 19970203
Entered Medline: 19920721

AB Control elements of the tetracycline-resistance operon encoded in Tn10 of Escherichia coli have been utilized to establish a highly efficient regulatory system in mammalian cells. By fusing the tet repressor with the activating domain of virion protein 16 of herpes simplex virus, a tetracycline-controlled transactivator (tTA) was generated that is constitutively expressed in HeLa cells. This transactivator stimulates transcription from a minimal promoter sequence derived from the human cytomegalovirus promoter IE combined with tet operator sequences. Upon integration of a luciferase gene controlled by a **tTA**-dependent **promoter** into a **tTA**-producing HeLa cell line, high levels of luciferase expression were monitored. These activities are sensitive to tetracycline. Depending on the concentration of the antibiotic in the culture medium (0-1 microgram/ml), the luciferase activity can be regulated over up to five orders of magnitude. Thus, the system not only allows differential control of the activity of an individual gene in mammalian cells but also is suitable for creation of "on/off" situations for such genes in a reversible way.

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